

# Characterization of a New Enterococcal Gene, *satG*, Encoding a Putative Acetyltransferase Conferring Resistance to Streptogramin A Compounds

Streptogramin antibiotics are mixtures of two chemically unrelated A and B compounds that act synergistically in vivo against gram-positive pathogens, such as staphylococci, streptococci, and enterococci (8, 11). Resistance against B compounds is very widespread among enterococci and is mediated via the *ermB* gene cluster (e.g., on Tn917) that confers macrolide-lincosamide-streptogramin B resistance (7). The synergistic mixture of streptogramins A and B overcomes resistance to B compounds but is inactive in resistance to A compounds. The only known resistance mechanism against streptogramin A compounds in enterococci is mediated by the streptogramin acetyltransferase *SatA* (9). *Enterococcus faecium* isolates with *satA*-mediated resistance have been found in samples of human and animal origins, indicating a possible spread of resistance genes or resistant bacteria among different ecosystems (10).

We isolated a quinupristin-dalfopristin-resistant *E. faecium* UW1965 from a sewage treatment plant in Germany. The resistance determinant was transferred to a susceptible recipient, producing the transconjugant UW1965K1. UW1965K1 is resistant to quinupristin-dalfopristin ( $\text{MIC} \geq 16 \mu\text{g/ml}$ ) and virginiamycin M (A compound;  $\text{MIC}, 16 \mu\text{g/ml}$ ), whereas the

MIC of each antibiotic for the recipient was 1 µg/ml. PCR amplification for the *satA* gene was negative.

In staphylococci, resistance to streptogramin A compounds is mediated by two mechanisms: (i) acetylation of the streptogramin A via acetyltransferases (*Vat*, *VatB*, and *VatC* [1-3]) and (ii) efflux due to an ABC transporter (*Vga* and *VgaB* [4, 5]). PCR amplification for the *vat*, *vatB*, *vatC*, and *vga* genes failed to produce any product. The putative protein sequences of the known streptogramin acetyltransferases in staphylococci and enterococci contain three conserved motifs (2). Corresponding primers, *satI* and *satJ*, have been made, producing a 144- to 147-bp fragment for *vat*, *satA*, and *vatB* (2). PCR performed with these primers resulted in a ca. 150-bp fragment for UW1965K1. A digoxigenin-labelled probe of the amplified fragment was prepared, hybridizing with a 5.5-kbp fragment of *Eco*RI-digested plasmid DNA from the transconjugant. The corresponding plasmid fragment was cloned into pUC18 and sequenced.

The resulting DNA sequence (Fig. 1) did not show significant identity with other gene sequences from GenBank on the DNA level (6). One suitable open reading frame (ORF) was found, giving rise to a putative 214-amino-acid (214-aa) pro-

1 cggatcccg ggatccctta gactataatt aaaattaaat aactcaattc ggaggtacta  
 start primor oto1  
 61 acgtgactat acctgacgca aatgcaatct atcctaactc agccatcaaa gaggttgct  
 M T I P D A N A I Y P N S A I K E V V F  
 aa-motif I  
 121 ttatcaagaa cgtgatcaa agtcccaata ttgaatttggg ggactacacc tattatgat  
 I K N V I K S P N I E Y G D Y T Y Y D D  
 181 acccgataaa tcccaccgat tttgagaac acgttaacc tcaatct attctaggcg  
 P V N P T D F E K H V T H Y E F L G D  
 241 acaattata catcgataaa tttgttctttcgccagtgg cattgaaattttatcatgaacc  
 K L I I G K F C S I A S G I E F I M N G  
 aa-motif II  
 301 gtgcccaaacca cgtataaa ggtatttcgcttatccatt taatattttta ggtggcgatt  
 A B H V M K G I S T Y P F N I L G G D W  
 361 ggcaacaata cacttctgaa ctgactgatt tgcgcgttgaa aggtgtact gtagtgtggat  
 Q Y T P E L T D L P L K G D T V V G M  
 aa-motif III  
 421 atgacgtgtg:gtttgggcaa aattgaccc tccattaccagg cgtaaaaata ggtgacggtg  
 D V W Y G N V T V L P G V K I G D G A  
 481 ccattatcg agcaaatagt gttgtaacaa aagacgtgc tccatataca attgtgcgtt  
 I I G A N S V V T K D V A P Y T I V G G  
 primor oto2  
 541 gcaatccat tcaactcattc ggaccaagat ttgaaccgga agtttatcaa gcattaaaa  
 N P I Q L I G P R F E P E V I Q A L E E  
 601 atctggcatat gtggaaaaa gatttgaaat ggttactgc taatgttct aaactaaatgc  
 L A W W N K D I E W I T A N V P K L M Q  
 stop  
 661 aaacaacacc caacattgaa ttgataacaa gtttaatgga aaaatataaa caaaaaagcc  
 T T P T L E L I N S L M E K \*  
 721 gtgcaagca tccaaaatat tgtttaca cggcctttac ttattgtga atccaattta  
 781 ttaataatag atatgtata ccagaaaa atacacatgc cacctctggc ggtactcta  
 841 tcgtatatttt tatttacgac ctttctgtatga taaaggtcac ttcccctgttccc ccaaaaaata  
 901 aaucc

FIG. 1. A 904-bp sequence located on the 5.5-kbp cloned fragment in pUC18 (GenBank accession no. AF139725). The ORF begins at nucleotide 63 with an ATG start codon preceding a putative ribosomal binding site (RBS) (double-underlined) at positions 50 to 57. The predicted gene sequence encodes a protein of 214 aa which shows significant homology with other streptogramin acetyltransferases (aa motifs I, II, III; see also Fig. 2). The locations of the primers satG1 and satG2, specific only for the *satG* sequence, are underlined (plus strand).

		<b>Motif I</b>		
SatG:	1	M-----TIPDANAIYPNSAIKEVVFIIKGNV-I-KSPNIEIGDYTYYDDPVNPNTDPEIGHVTHHYEPLGDKL 63		
VatB:	1	MK----YGPDPNSIYPHEEIKSVCPIKNTI-TNPNIIVGDTTYYSDVNNGAEKPEEVVTMRYEFRGDKLV 64		
VatC:	1	MKWQNQQGPNEEIYPICGNKHVQPIKPSI-TKPNLIVGEYSYTDISK-DGESPESQVLYHYLEIGDKL 67		
SatA:	1	M-----GPNPNDHYPIEGNKSVPQIKPILEKLENVEVGKSYTDISK-NGETPDKQILYHYPILNDKLK 62		
Vat:	1	MNLNNNDHGPDPENILPIKGNRNLQPIKPTI-TNENILVGEYSYTDISKRGES-FEDQVLYHIVETIGDKL 67		
		***	***	***
		<b>Motif II</b>		<b>Motif III</b>
SatG:	64	IGKPCSIASGIEFIMNGAMEVMIKGISTYPPNILGGDWQOYTPEL-TDLPLKGDTVVGMWDWVPGQNV 128		
VatB:	65	IGKPCAIAGIEFIMNGAMERNSITTYPPNIMGNOWEKATPSL-EDLPPKGDTVVGMWDWVPGQNV 129		
VatC:	68	IGKPCSIGPGTTFIMNGAMERMDG-STPPNLLPGNGWEKHTPTL-EDLPYKGNTIEIGMWVIGRDV 131		
SatA:	63	IGKPCSIGPGTTFIMNGAMERMDG-STPPNLLPGNGWEKHMPL-DOLPIKGDTIIGMWVIGRDV 126		
Vat:	68	IGRCPCSIGPGTTFIMNGAMERMDG-STPPFLFRMGWEKYMPSL-KDLPLKGDIEIGMWVIGRDV 131		
		***	***	***
SatG:	129	TVLPGVKIGDGAIIAGANSVVTKDVAAPYTIVGGNPQLIGPRFEPEVIQALENLAW 183		
VatB:	130	TVMPGQIIGDGAIVAAANSVVTKDVPYRIIGGNSPRIKKRFEDELIDYLICIKW 184		
VatC:	132	TIIMPVGKIGDGAIIAAKSVVTKNDVDPYSVVGNPSRLIKIRFSKKEIAALLKVRW 186		
SatA:	127	VIMPVGKIGDGAIVAAANSVVKDIAPIYMLACGNPANEIKQRFDQDTINQLLDIKW 181		
Vat:	132	TIIMPVGKIGDGAIIAAEAVVTKNVAPYSTVCGNPLKIRKRFSDGVIEEWLALCW 186		
		***	***	***
SatG:	184	WNKDIEWITANVPKLMOTTPTLELINSMEK 214		
VatB:	185	WDWSAQKIFSNLETLCSS--DLEKIKSIRD 212		
VatC:	187	WDLEIETINENI 198		
SatA:	182	WWNPIDIIENIDKILDNSIIIREVI 206		
Vat:	187	WNLOMKIINENLP 199		

FIG. 2. Alignment of amino acid sequences of acetyltransferases from staphylococci and enterococci (1–3, 9) conferring resistance to streptogramin A antibiotics. Identical residues are indicated by asterisks. Highly conserved regions in different streptogramin A acetyltransferases—motifs I, II, and III—are boldfaced. Primers sat1 and satJ have been designed on the basis of the corresponding nucleotide sequences in motifs II and III (2).

tein. A comparison of amino acid similarities indicated rather significant homology between streptogramin acetyltransferases and the new putative acetyltransferase, designated SatG (Fig. 2). Based on the sequence for *satG*, two primers, satG1 and satG2, have been designed. Preliminary results of a search for streptogramin-resistant enterococci (*E. faecium*, *E. hirae*, and *E. durans*) revealed the existence of the *satG* gene in 9 of 23 isolates from sewage, 6 of 24 isolates from broiler samples, and all 17 isolates from poultry manure. Of 62 quinupristin-dalfopristin-resistant *E. faecium* (QDREF) isolates from hospitals in Germany, 9 were positive for *satG*. The high number of *satG* QDREF isolates from poultry meat and manure may be due to selection of these bacteria by use of virginiamycin as a feed additive, and spread of the resistance via the food chain to humans is very likely. This hypothesis is being investigated.

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